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LSD and Genetic Damage

Is LSD chromosome damaging, carcinogenic, mutagenic, or teratogenic?

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Chromosome damage in human white blood cells, leukemia, malformed infants, and animal mutations have been reported in man and other organisms that had been exposed to lysergic acid diethylamide (LSD). In the past 4.0 years, 68 studies and case reports directly related to this issue have been published. We have undertaken to review these studies in order to clarify what is now known, and to help resolve the problems relating to the use of this drug. The questions we attempt to resolve are whether LSD is a chromosome-breaking agent and whether it is a carcinogen, a mutagen, or a teratogen in man.

Chromosome Studies in vitro

In our discussion, terms such as "chromosome breakage" and "chromosome damage" include both chromatid-type and chromosome-type aberrations. When necessary, or if permitted by the data reviewed, we distinguish between these two quite different types of aberrations.

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Cohen and associates (*1*) added LSD to cultured human leukocytes in five concentrations ranging from 0.001 to 10.0 micrograms of LSD per milliliter for 4, 24, and 48 hours each. The breakage rate for treated cells was at least twice that of control cells for all treatments, except at the lowest concentration and time (0.001 μg of LSD per milliliter for 4 hours) where no difference existed between treated and control cells. The frequency of aberrations, however, was not related simply to dosage or duration of exposure.

Loughman *et al.* (*2*) emphasized that it is precisely the lowest concentration and duration of exposure that most closely approximates the expected concentration in blood, liver, and other organs after a dose of 100 μg of LSD ingested by a man weighing 70 kilograms. For example, the half-time of LSD in plasma is approximately 175 minutes in mice (*3*) and humans (*4*) and thus the mean circulation time is about 4 hours. A 100-μg dose of LSD uniformly distributed in a 70-kg man will yield a uniform concentration of 0.0014 μg/g. The calculated expected concentration in blood after 30 minutes is 25 percent of this value, or 0.0004 μg/g. If the metabolic degradation of LSD is considered (*3*), then the effective concentration in vivo of unchanged LSD would be approximately 0.0001 μg/ml—a concentration used only by Kato and Jarvik (*5*), who found no increase in breakage at this dosage.

Experiments in vitro on a variety of cell types have been reported by eight teams of investigators (Table 1). Chromosome damage has been reported (*1, 5–9*), although not consistently (*10–12*), at concentrations of LSD and durations of exposure greater than 0.001 μg/ml for 4 hours. In repeating their work Cohen *et al.* (*6*) now have found significantly increased rates of breakage at this lowest concentration and duration of exposure. Their finding has not been confirmed by Tjio (*8*).

Interpretation of the significance of chromosome damage in vitro requires the following considerations:

1) In all of the studies on cultured lymphocytes a modification of a technique (*13*) was used in which phytohemagglutinin stimulates lymphocytes to enter the reproductive cell cycle. In the normal state in vivo small lymphocytes are in the G_1 phase of growth, which precedes DNA synthesis. They do not grow, or divide, or enter the cell cycle (*14–16*). Thus, in the studies in vitro, lymphocytes are exposed to chemical agents during developmental stages of the cell cycle, including DNA synthesis, which do not normally occur in these cells in the body. Damage to a lymphocyte in the G_1 phase generally will not manifest itself as a chromatid-type change in a subsequent division (*15, 17*). Most, if not all, chromatid-type changes are initiated by technical procedures (*15*). The great majority of lesions reported in most studies in vitro and in vivo were of the chromatid type; therefore, the findings of increased rates of chromosomal breakage in lymphocytes exposed to LSD in vitro must be interpreted with great caution.

2) This need for caution is well illustrated by the large numbers of conditions and substances commonly used which have been reported to induce chromosomal breakage in vitro and which include changes in temperature,

changes in oxygen pressure, the nucleotides, antibiotics, benzene, caffeine, calcium and magnesium deficiencies, chloroform, mercury compounds, morphine, theobromine, theophylline, water unless twice distilled, and many others (*18*). An example may be derived from the study of Kato and Jarvik (*5*) who examined lymphocyte chromosomes for breakage rates induced by LSD, acetylsalicylic acid (aspirin), and ergonovine (lysergamide) maleate (a widely used oxytocic). Each compound doubled the breakage rate in control cells and appeared equally effective in producing breaks (10.2, 9.6, and 10.0 percent, respectively). Breakage of chromosomes induced by aspirin in vitro has been supported by one study (*19*), but not by another (*20*) in which experiments in vitro and in vivo were performed.

3) The intact human organism differs from cells in the test tube in vitro in its ability to detoxify and excrete noxious compounds. Compounds that are toxic in vitro do not necessarily have the same effect in vivo.

In summary, no consistent dose-response relation has been reported. Generally, damage occurred with high concentrations or prolonged exposures (or both) which could not be achieved in humans given reasonable doses. The magnitude of breakage, when found, was within the range induced by many agents in common usage. We believe that the special nature of the test system in vitro, the several negative reports, and the absence of excretory and detoxification mechanisms all suggest that the results obtained in vitro would inadequately predict the effects of LSD exposure in man.

Chromosomal Studies in vivo

Does ingestion of LSD by humans produce chromosome damage? If so, is the damage related to carcinogenesis or genetic damage in subsequent generations? Many of the 21 reports from 17 laboratories are accounts of experiments in vivo that are more or less inadequate. As a result, individual reports have been contradictory and at best inconclusive. However, consistent trends do emerge from the evidence examined.

Two types of experimental design have been used. In 11 of the studies, LSD groups consisted of individuals exposed to known quantities of pure LSD in experimental settings. In 14 studies, subjects were exposed to illicit substances alleged to be LSD, but which were of unknown composition and potency. The significance of distinguishing between medically supervised groups treated with pure LSD in contrast to groups exposed to illicit LSD was suggested by several reports that showed marked discrepancies between alleged and actual composition and potency of illicit drugs (*21–24*).

Of 310 subjects that have been studied (Tables 2 to 4), 126 were treated with pure LSD. The other 184 were exposed to illicit, "alleged" LSD. A maximum of 18 of 126 (14.29 percent) of the subjects in the group given pure LSD showed a higher frequency of chromosome aberration compared to that of the controls (means). In contrast, a maximum of 90 to 184 (48.9 percent) of the subjects in the group taking illicit LSD showed an increased frequency of aberrations. The frequency of individuals with chromosome damage reported among illicit drug users is more than triple that for subjects given pharmacologically pure LSD. Of all the subjects reported to have chromosome damage, only 16.67 percent (18 of 108) were given pure LSD.

Illicit LSD and Chromosome Damage

Irwin and Egozcue (*25, 26*) reported the initial findings of chromosomal damage in illicit LSD users. In the first (*25*) of two reports, illicit LSD users had a mean breakage rate of 23.4 percent, nearly double the 11.0 percent rate in controls who did not use drugs. Only two of the eight users cited did not have increased breakage rates. In a subsequent and more extensive study (*26*), the mean breakage rate in 46 illicit LSD users was 18.76 percent (range 8 to 45 percent); this was double the rate of 9.03 percent found in control cells. Only three of the 46 users did not have a breakage rate higher than the mean control rate. Of four children exposed in utero, one did not have elevated breakage rates. Irwin and Egozcue emphasized that the effect was long lasting (up to 2 years) in these children, but there was no evidence of ill health or physical malformation in any of them.

The above-mentioned findings were supported by Cohen *et al.* (*6*) who reported that 18 subjects exposed to illicit LSD had an elevated chromosome breakage rate that was triple the mean of 3.8 percent for the control group. All subjects exposed to

Table 1. Chromosomal studies in vitro. Chromosome studies in vitro are designated by reference number. They are arranged according to reported results: Plus sign, chromosomal breakage rate of LSD-treated cells significantly elevated above control rate; minus sign, no significant elevation of breakage rate. Some studies are cited twice in order to present both the negative and positive findings.

Study		Cell type	LSD (μg)	Exposure (hr)	Treated cells		Control cells	
Ref.	Result				No.	Breaks (%)	No.	Breaks (%)
(*1*)	+	Lymphocyte	0.001 to 10	4–48	2678	6.7–36.8	925	3.7
(*5*)	+	Lymphocyte	0.01	4–24	598	10.2–12.0	100	5.0
(*6*)	+	Lymphocyte	0.001 to 10	4–48	8725	7.7–17.5	1680	3.9
(*7*)	+	Lymphocyte	1.0	24	1010	4.0–18.7	1094	0.0–15.1
(*8*)	+	Lymphocyte	0.001 to 10	48–72	2750	10.8–72.4	500	5.2–7.6
(*9*)	+	Barley root	25	4–8	791	37.7–56.2	470	0.0–1.6
(*1*)	–	Lymphocyte	0.001	4	200	5.0	925	3.7
(*5*)	–	Lymphocyte	0.0001 to 1.0	4–24	600	2.0– 5.0	100	5.0
(*8*)	–	Lymphocyte	0.001 to 10	4	1250	4.8– 8.4		
			0.1 to 10	48	750	8.0– 9.6	500	5.2–7.6
			1.0	64	250	8.8		
(*10*)	–	Lymphocyte	0.01 to 0.1	48	453	7.2– 8.3	184	4.3
(*11*)	–	Lymphocyte	10.0	24–48	372	0.3	329	0.0
	–	Fibroblast*	10 to 20	2–48	850	0.1	400	0.0
	–	*Vicia faba*	1.0 to 50	2–24	300	0.0		
(*12*)	–	Embryo*	0.9 to 45	24–72	274	0.0		

* Hamster.

illicit LSD had breakage rates that were higher than the mean for the control group.

Cohen *et al.* (*27*) reported that 13 of the adults exposed to illicit LSD had chromosome breakage rates above the mean of the controls. In nine children exposed to illicit LSD in utero the mean breakage rate was 9.2 percent compared to 4.0 percent in four children whose mothers had used illicit LSD before but not during pregnancy. The control rate was 1 percent. Here, too, Cohen *et al.* emphasized the apparent long-lasting effect suggested by the findings. All but two children had been exposed to other drugs during gestation, all were in good health and exhibited no birth defects.

Nielsen *et al.* (*28*) found that ten subjects exposed to illicit LSD had a mean breakage rate (2.5 percent) that was higher than that of the controls (0.2 percent). However, the 2.5 percent rate was lower than that of the controls in the other positive studies, and the number of subjects with higher breakage rates was not given. In all of the positive studies there was no correlation between dose, time since last exposure, number of exposures, or frequency of aberration.

Nine groups of investigators have been unable to support the positive findings (*2, 29–36*) (Table 2). In three studies (*30–32*) karyotypes were constructed to detect the presence of translocations, deletions, Ph[1]-like chromosomes, and other morphological changes. None was found. In another report (*33*) autoradiographic studies of cells from 20 exposed individuals showed no difference in labeling pattern from controls.

In several of the negative studies an attempt was made to resolve the discrepancies between positive and negative reports by criticizing the breakage rate for controls indicated by Cohen *et al.* (3.8 percent) and by Irwin and Egozcue (11.9 and 9.03 percent) as being unusually high (*2, 5, 29, 48*). Extensive studies of normal populations have provided both low and high control rates (*17, 37–40*) (Table 3). It was suggested that the high control values may have arisen from suboptimum conditions of cell culture (*2*), use of less-than-complete culture medium (*29*), viral contamination of culture (*5*), or artifacts arising from micromethods of culture (*48*). In the studies of Cohen *et al.* and of Irwin and Egozcue most of the described aberrations were chromatid-type changes. Chromosome-type and chromatid-type changes were not reported separately but were combined and then converted to "equivalent numbers of breaks." Combining the two types of aberrations in a single index obscures the distinction between real chromosome damage occurring in vivo and damage arising in the course of cell culture. Aberrations resulting from these effects should be distributed randomly between groups exposed to illicit LSD and control groups, but they are not. Therefore, these factors do not explain the nonrandom, significantly elevated breakage rates in 80 of 86 subjects exposed to illicit LSD studied by Cohen *et al.* and by Irwin and Egozcue.

In the foregoing studies, all the subjects used illicit LSD. To ascertain the patterns of drug use the experimenters had to rely on the subjects' recall and reliability; and, as a result, dosage, frequency, purity, total num-

Table 2. Chromosomal studies of illicit LSD users. Groups exposed to illicit LSD and other black-market drugs were compared to unexposed controls. Children exposed in utero are not included. Studies are designated by reference number. They are arranged according to positive (+) or negative (−) findings, and then in order of increasing percentage of the breakage rate as compared to that of the controls; WNL, chromosome breakage rate within normal limits, percent not given.

Study		Illicit LSD groups			Controls			Time from last LSD (months)
Ref.	Result	Subject (No.)	Cells (No.)	Breaks (%)	Subject (No.)	Cells (No.)	Breaks (%)	
(*2*)	−	8	697	0.0	19	673	0.2	0.033–0.75
(*35*)	−*	14	1412	0.36	8	805	0.63	0.25–47
(*30*)	−	17	595	1.01	8	280	0.72	0.25–6
(*32*)	−†	14	1284	0.76	10	1018	0.79	0.002–3
(*29*)	−‡	4	937	1.4	4	950	1.5	0.25–6
(*34*)	−	3		1–4.0	11		4.7	
(*36*)	−	5		WNL				
(*31*)	−	3	350	WNL	4			5–7
(*33*)	−	20		WNL				
(*28*)	+	10	635	2.5	41	1584	0.28	
(*27*)	+§	14		7.53	9		1.20	0.25–30
(*6*)	+‖	18	4282	13.2	12	2674	3.8	0.5–8
(*26*)	+	46	9140	18.76	14	2800	9.03	0.033–12
(*25*)	+	8	1600	23.4	9	1800	11.9	0.033–6

* Chlorpromazine control group: two subjects, 200 cells, 1.5 percent breaks. † Also includes a group exposed to cannabis: nine subjects, 816 cells, 0.86 percent breaks. ‡ Includes combined results of two independent laboratories. § Does not include three adults previously reported in (*6*). ‖ Control breakage rate does not include two subjects retrospectively eliminated because of viral infection. Breakage rates were 31.0 and 14.0 percent. Also includes a control group that used drugs but not LSD. Six subjects, 1361 cells, 12.6 percent breaks.

Table 3. Chromosomal studies of users of pure LSD. Comparison of groups treated with pure LSD to unexposed controls. Studies are designated by reference number. They are arranged according to positive (+) or negative (−) findings, and then in order of increasing percentage of the control breakage rates. The blood sample was taken after the last exposure to LSD (months).

Study		Pure LSD groups			Controls			Time from last LSD (months)
Ref.	Result	Subject (No.)	Cells (No.)	Breaks (%)	Subject (No.)	Cells (No.)	Breaks (%)	
(*35*)	−	5	500	0.40	8	805	0.63	0.1–102
(*47*)	−*	22	2200	0.86	32	3200	0.66	24–48
(*29*)	−†	4	914	1.4	4	950	1.5	1–60
(*46*)	−	5	50	< 2.0	5	50	< 2.0	20–48
(*48*)	−	8	1646	2.79	2	400	2.65	2.2–14.6, 9–163
(*7*)	−	11	1094	7.36	13	1300	7.0	0.33–96
(*44, 45*)	±‡	5	358	1.70	23	802	0.00	6–38
(*28*)	+§	9	603	4.30	11	554	0.5	
					30	1030	0.2	
(*48*)	+	1	200	12.00	6	925	3.7	8
(*50*)	+‖	1	300	3.5	3	900	1.2	
(*37*)					20	1810	0.0	
(*38*)					417	12,400	0.4	
(*17*)					11	1569	0.7	
(*40*)					171	10,393	1.7	
(*39*)					10	3720	7.4	

* Pure LSD group does not include one subject with 9 percent breakage rate excluded by reason of being widely deviant. † Includes combined results of two independent laboratories. ‡ Includes a group (*44*) treated with psychotropic medications. There were 17 subjects, 510 cells, and 1 percent breaks. Control group (*45*) contains 40 subjects (23 drug-free controls and 17 patients treated with psychotropic medication); 1312 cells and 0.4 breaks. § Also includes a phenothiazine-treated group; there were 28 subjects, 1841 cells, and 1.4 percent breaks. ‖ Subject not included in calculations because of prior extensive therapeutic radiation.

Table 4. Chromosomal studies of pure LSD users studied before and after they took LSD. Each subject was tested before and after treatment with pure LSD. Studies are arranged in order of increasing differences in breakage rates before and after treatment. Except for (*50*) differences were not statistically significant. Most subjects received single treatments, five subjects from (*48*) and three from (*50*) received up to three treatments. Administration of LSD was oral, except in (*50*) where it was intravenous. Of the six subjects with significantly elevated breakage rate, five were tested again 2 weeks to 7.67 months after last treatment. In all five cases breakage rate returned to that before LSD was taken.

Study		*N*	Cells (No.)	Breaks		Difference	Time from last LSD (hours)	Single LSD dose (μg)
Ref.	Result			Before LSD (%)	After LSD (%)			
(*7*)	−	10	1000	5.7	4.9	−0.80	24	200–600
(*49*)	−	6		0.0	0.0	0.0		65–300
(*48*)	−	5	7600	2.81	3.57	+0.76	0.5–48	100–150
		32	14,984	4.28	5.91	+1.63	24–240	50–450
(*50*)	+	3	1725	1.0	4.78	+3.78	1–336	187–200

ber of exposures, and interval since last exposure were merely estimations.

It is likely that drugs used by these individuals contained impurities. Marshman and Gibbins found wide discrepancies between the alleged and actual composition of illicit drugs (*21*). Only 54 percent of 57 samples alleged to contain LSD actually contained this drug in a relatively pure form. The remainder contained a large proportion of impurities or no LSD at all. The laboratory of the Federal Bureau of Narcotics and Dangerous Drugs in San Francisco has found DOM (4-methyl-2,5-dimethoxyamphetamine, also called STP) and PCP (phenylcyclohexylpiperidine) either substituted entirely for LSD or added to samples containing LSD (*22*).

Krippner (*23*) analyzed 12 tablets (from various sources) alleged to contain 250 μg of LSD. One contained no LSD; most contained very small quantities (2, 7, or 26 μg); some contained more DOM than LSD; only two contained more than 150 μg of LSD. Discrepancies of comparable magnitude in illicit LSD samples were also reported by Cheek, Newell, and Joffee (*24*).

Thus the estimated LSD dosages obtained from interviewing subjects who used illicit LSD are unreliable. Nearly all the subjects used or abused drugs other than LSD. These drugs included alcohol, amphetamine, antihistamines, aspirin, barbiturates, cannabis, cocaine, diethyltryptamine, dimethyltryptamine, DOM, heroin and all other varieties of opiates, mescaline and the methylenedioxy amphetamines (MDA, MMDA), nicotine, peyote, phenothiazines, psilocybin, and ritalin.

The role of other chemical agents was illustrated by Cohen *et al.* (*6*) who compared a group of six individuals ingesting drugs other than illicit LSD (chlorpromazine, amphetamine, barbiturates, cannabis, and diphenhydramine) with 18 subjects exposed to illicit LSD along with other drugs. The mean chromosome breakage rate of 12.6 percent for the former group was not significantly different from the rate of 13.2 percent for the latter.

Breakage rates higher than the mean rates for controls were found in a maximum of 90 of the 184 adults (less than half) exposed to illicit LSD. Of these adults, detailed drug histories were available on 77, of whom 43 (56 percent) used amphetamine. In our experience, abusers of amphetamine are the most physically debilitated of those who abuse drugs. Of all illicit drugs, methamphetamine is reputed to be especially contaminated and toxic; this impression has been substantiated. Residues of three solvents—benzene, ether, and chloroform—used in the manufacture of methamphetamine, as well as dangerous unreacted raw materials such as mercury, phenyl-2-propanone, and methylamine have been found in illicit samples of this drug (*22*). Material sold as liquid methamphetamine was found to contain human urine and a toxic floor cleaner (*21*).

We believe that the initial sample populations (*6*, *25–27*) were inadequately described as "LSD users." In fact, most subjects were multiple drug users, abusers, or addicts exposed to toxic substances of unknown composition and potency. In view of the unreliability of self-report and the unpredictable composition of illicit drugs, it is unlikely that all investigators could have sampled comparable drug-abuse populations.

Smith and Rose (*41*) have reported very high rates of hepatitis and other viral illness among drug abusers in San Francisco. The role of viral infection, which has been reported to induce chromosome damage (*42*), was not independently assessed in the positive studies. The role of malnutrition, a predisposing factor to infection and a common condition among amphetamine abusers, remains to be evaluated.

The numerous methodologic questions that have been raised here certainly qualify but do not completely dismiss the unusually high frequencies of chromosome aberration reported by three separate teams of investigators. It could well be that a combination of factors such as long-term excessive exposure to illicit chemical agents, the presence of toxic contaminants, the intravenous route of administration, or the physical debility of many drug abusers would result in chromosome breakage to the extent reported. However, the early reports of three laboratories were apparently based on studies of drug-abuse subjects who did have excessive chromosome breakage. Nine teams of investigators independently studying similar individuals not only failed to substantiate these earlier findings but were able to demonstrate only a single instance (an infant exposed in utero) (*31*) of chromosome damage beyond that present in the general population. Incomplete data on ten subjects (*28*) precludes calculation of a precise percentage of subjects with elevated breakage rates. However, this figure would range between 48.91 percent (90 of 184) and 45.98 percent (80 of 174). We conclude that positive results, when found, are related to the more general effects of drug abuse and not, as initially reported, to LSD use.

Pure LSD and Chromosome Damage

When chromosomal studies of users of pure LSD were made, the potency, purity, and frequency of exposure to LSD did not depend on the subject's presumed knowledge or recalled estimates. The interval between exposure and blood sampling was under direct experimental control. For these reasons, the studies of users of pure LSD provide more reliable evidence concerning the questions of whether LSD damages human chromosomes in vivo and, if so, whether this damage is of a transitory or relatively permanent nature.

The studies of pure LSD users can be divided into two classes. Most studies compared a group that had been ex-

posed to LSD with a control group that had not been exposed either to LSD or to any other drug. For convenience and clarity of description, this type of experiment will be referred to as experiment 1, which is distinguished from experiment 2 in which each subject studied before and after use is used as his own control.

Chromosome Studies after LSD Treatment

In their initial study Cohen *et al.* (*1*) reported that chromosomal damage in white blood cells of humans treated with pure LSD was observed in one paranoid schizophrenic individual treated 15 times with 80 to 200 μg of LSD over a period of 5 years. The damage found was a chromatid exchange figure and a significant increase in breakage. Cohen *et al.* suggested that chromosome damage found 8 months after the last LSD treatment was analogous to the chromosome damage of long duration induced by radiation and associated with leukemia (*43*) and that their findings must be interpreted with caution because the patient had been treated with the phenothiazine chlorpromazine and chlordiazepoxide until 8 months before the study.

A review of the chromosomal studies of the experiment-1 type has revealed that only two groups of investigators reported that their subjects showed an increased rate of chromosome breakage (12 of 70; that is, 17.1 percent) (*1, 28, 44, 45*). Five other teams of investigators (*7, 29, 35, 46–48*) failed to confirm these earlier reports and were able to demonstrate only one doubtful instance of chromosomal damage beyond that present in the general population (*47*) (Table 3).

Nielsen *et al.* studied five subjects treated with pure LSD and found "no correlation between any specific drug and the frequency of gaps, breaks, and hyperdiploid cells" (*44*). They later (*45*) regrouped their original data (same subjects, cells, and aberrations) and concluded that LSD induced chromosome damage. Five individual aberrations initially tabulated in a group treated with phenothiazines were reassigned to different groups when the control subjects were reclassified, smaller groups thus being formed, on the basis of age and sex. In addition, Tjio *et al.* have emphasized that the number of cells analyzed (71.6 per LSD subject and 34.4 per control, on the average) was insufficient to determine breakage rates reliably. This variable is particularly relevant because of the small number of lesions identified. Three of the five LSD subjects had no aberrations. Two subjects accounted for the six breaks found. The 1.7 percent breakage rate is well within the values reported for the general population (Table 3).

In a second study by Nielsen *et al.* (*28*) the methods used to count and analyze data were unusual, as follows: (i) In tabulating exchange figures and fragments, the pure LSD subjects were combined with subjects exposed to illicit LSD; intergroup comparisons were thus impossible. (ii) Dicentrics, rings, acentric fragments, and centric fragments were scored separately. Damage that produces dicentrics and rings simultaneously produces acentric fragments. Therefore, the aberration rate may have been inflated, since two-thirds of the chromosome-type lesions found in the combined LSD group were acentric fragments. (iii) As few as two or three patients with markedly elevated breakage rates could account for all the breakage found. Also, the actual number of treated patients with breakage rates above controls was not given.

In summary, 82.9 percent (58 of 70) of the subjects studied after treatment with pure LSD did not have chromosome damage. Because of incomplete data (*28*) on nine of the remaining 12 subjects, it is not possible to compute the precise percentage of subjects with elevated breakage rates. However, this figure would range between 17.1 percent (12 of 70) and 4.9 percent (3 of 61). All but one of the 12 were reported by a single team of investigators (*28, 44, 45*). In view of the procedures, incomplete data, questionable reanalysis of the data, and low breakage rates reported, we conclude that there is no definite evidence from type-1 experiments (that is, studies of individuals after they had been treated with pure LSD) that pure LSD causes chromosome damage.

Before and after LSD Use

Tjio *et al.* (*48*) reported a well-designed study in which more than 22,500 cells from 37 patients were examined before and after treatment with LSD. The number of cells observed was more than twice the total number of cells observed in all other studies of pure LSD users. The effects of both single and multiple doses were examined. In the study on the effects of a single treatment with 50 to 450 μg of LSD, the baseline breakage rates before exposure were established 1 to 66 days before the drug was given. The rates after exposure were established 1 to 10 days after treatment. There was no significant difference in LSD breakage rates in either high or low dose groups. There was no relation between dose and amount of breakage. On the contrary, those in the low dose group showed greater increases after treatment than those on high dose. Another group was composed of five persons who had taken illicit LSD from 4 to 36 times before the study. In this series the investigators took blood cultures for seven to ten consecutive days before, during, and after treatment with pure LSD at doses of either (1 μg or 2 μg/kg) two or three times. Again, there were no significant differences after the drug was taken.

These findings have received support from two other studies of the same kind (*7, 49*). Of the 53 individuals who ingested pure LSD (three studies), 50 (94.3 percent) showed no increase in breakage rates (Table 4).

In contrast, Hungerford *et al.* (*50*) observed an increase of chromosome aberration after each of three intravenous injections of LSD. This is the only study of intravenous administration. The increase in aberrations was small in two of the three subjects; however, dicentric and multiradial figures appeared only after treatment, and acentric fragments appeared more frequently after treatment. In samples of blood taken 1 to 6 months after the final dose, the breakage observed was apparently equivalent to that before LSD was taken.

Transitory effects after multiple, subcutaneous injections in rhesus monkeys, of high doses (125 to 1000 μg/kg per injection) of LSD were also reported (*51*); but a statistical evaluation was not provided, and our analysis indicates that there were no statistically significant positive effects.

In summary, only six of the 56 patients (10.7 percent) studied before and after treatment with pure LSD had elevated breakage rates; of these, three received LSD intravenously (*50*) and one had a viral infection (*48*). Of these six subjects, one individual was not available for follow-up determinations, but in the remaining five, the breakage returned to that observed before treatment, an indication of reversibility which could be, as was sug-

Table 5. Teratogenesis: rodent studies. Studies are arranged according to species and then in order of positive (+) or negative (−) results. The data was not presented in a uniform manner by all studies. Conversion from number of animals to percentage of animals possibly entailed minor distortions but did facilitate comparisons between studies. Dead includes stillborn or resorption; Off/Lit, ratio of the number of offspring to the number of litters; Mal, malformed animals.

Study		LSD (μg/kg)	Day	Treated animals				Controls			
				Off/Lit	Percentage			Off/Lit	Percentage		
Ref.	Result				Mal	Dead	Runts		Mal	Dead	Runts
				Mice							
(*69*)	+*	0.05–1.0	6–7	158/20	57			64/9	7.8		
(*70*)	+†	5	6–9	120/18	62.5			241/28	0.0		
(*12*)	+‡	0.5–30	6–7	79/14	19	3.8		66/10	3.3		
(*12*)	−‡	0.5–30	6–7	167/22	0.0			58/6	0.0		
(*71*)	−	5–500	4–14	521/67	0.0	20.7		70/10	0.0	35.7	
				Wistar rat							
(*76*)	+	5	4	85/10	0.0	23.6	3.5	130/10	0.0	0.0	0.0
(*77*)	+§	5–20	1–4	521/55		11		612/53		2	
(*80*)	±‖	2–6 × 10^3	4	594/52	0.0	3.4	0.5	365/29	0.0	0.8	0.3
(*76*)	−	5	7–16	51/5	0.0	0.0	0.0	65/5	0.0	0.0	0.0
(*78*)	−¶	1.5–300	4–12	887/89	0.5	5.9	0.0		1.0		
(*79*)	−	2.5–10	4	666/49	0.0	0.2	1.2	390/29	0.0	0.5	0.5
(*71*)	−	5–500	4–13	1003/98	1.0	14.8		203/20	0.0	25.7	
				Hamster							
(*81*)	+**	0.84–240	8	378/37	5–8	11.7	8.5	300/25	0.0	1.0	1.0
(*12*)	−	10–300	6–9	171/14	0.6	1.8					
(*71*)	−	50–500	4–13	189/22	0.0	31.2		170/18	0.0	22.4	
				New Zealand White rabbit							
(*82*)	−	100–300	4–12	123/14	0.0	4.1		45/6	0.0	6.7	

* Animals treated on days 8 to 9 had normal offspring. † 58 to 81 percent lens abnormalities, but no CNS anomalies. Offspring of animals treated on days 4 to 5 were normal. ‡ Data on hamster controls not given, but treated animals did not vary significantly from untreated. § Percent malformed not given, but no difference existed between treated and untreated animals. ‖ Negative for teratogenic effect, but LSD at 4760 μg/kg, 50 percent of females produced no viable births. ¶ Control data not given but treated animals did not vary significantly from controls, that is, 5.9 percent resorption rate for treated animals is within normal. ** 5 to 8 percent malformations. The total number of malformed animals was not given; the data also include 8 to 14 percent resorptions in treated as compared to 2 percent in untreated animals.

gested by Hungerford, attributable to a built-in mechanism for repair or elimination of aberrations.

Of the subjects studied before and after treatment, 89.3 percent did not have chromosome damage. This confirms the conclusions of five of seven teams that studied subjects only after treatment. Hence, we conclude that the ingestion of moderate doses of pure LSD does not break human chromosomes.

Is LSD a Carcinogen?

Cohen *et al.* (*1*) first suggested the carcinogenic potential of LSD. Their speculation was based on finding a quadriradial chromosome exchange figure and a markedly increased frequency of breakage. Such findings are associated with three inherited disorders in which there is a high incidence of leukemia and other neoplasia (*52*). The cause of the chromosomal lesions in these disorders is not known, nor is it known whether the chromosomal lesions have any relation to subsequent neoplastic development. Moreover, there are many chromosome breaking agents which are not associated with leukemia; indeed, no cause and effect relation has been demonstrated and none is known. Quadriradial and other rearrangement figures have been found in white blood cells of normal individuals (*2, 38, 39, 53*).

Cohen *et al.* (*1*) also suggested that the broken and rearranged chromosomes found in one patient 8 months after the last LSD treatment were analogous to the chromosome damage of long duration induced by radiation and associated with leukemia. The findings of long-term damage have been supported by three retrospective studies (*25–27*). In two reports of subjects studied before and after they took LSD (*48, 50*), the occasional damage that was found was without exception transitory, suggesting a reversibility of effect unlike that associated with radiation.

Supporting the carcinogenic hypothesis, Irwin and Egozcue (*25, 26*) reported that nine illicit LSD subjects had centric fragments resembling the Ph^1 chromosome often associated with chronic granulocytic leukemia. Grossbard *et al.* (*54*) reported a Ph^1-like chromosome in all 35 peripheral leukocytes examined from an individual who used illicit LSD and other illicit drugs and who did have acute leukemia. There was no other indication of chromosome damage in peripheral cells. We are not aware of other reports of leukemia in illicit LSD users, and we do not find other reports of a Ph^1-like chromosome in subjects exposed to pure or illicit LSD. In both studies the Ph^1-like chromosome was found in peripheral leukocytes. However, in chronic granulocytic leukemia, the Ph^1 chromosome is only characteristic of myeloid and erythroid cells, which normally do not divide in peripheral blood. Hungerford, who along with Nowell (*55*), initially described this lesion, wrote, "A chromosome compatible with the Ph^1 would have to be observed in blood cells other than lymphocytes to be relevant to the question of chronic granulocytic leukemia" (*56*), and ". . . in the absence of appropriate retrospective data concerning cancer patients and data concerning carcinogenic effects of LSD in experimental animals, the suggestion remains highly conjectural" (*50*).

Two cases of leukemia in individuals treated with pure LSD have been reported. In one (*57*) there was a ". . . remarkable incidence of childhood malignancies . . . strongly suggestive of a familial predisposition to malignant disease" (*57*). In the second, no details were given (*58*). Information relating LSD to leukemia is rare; as of now, there appears to be no definite evidence that LSD is a carcinogen.

Is LSD a Mutagen?

Mutagenesis has been widely studied in drosophila. Radiation and chemically induced mutations were initially detected in this organism (*59*). Grace

et al. (*60*) performed genetic tests of LSD for sex-linked lethals (a technique uniquely sensitive to mutagenesis), sex-linked recessive visibles, translocations, and a specific visible. Treated flies received intraabdominal injections of solutions of 1 to 500 μg of LSD per milliliter. Translocations were not present, and there were no significant differences between treated and control populations. The authors concluded (*60*), "LSD, if it is a mutagen or radiomimetic agent in human chromosomes, . . . is not a very powerful one. It is more probable that LSD induces neither mutation nor chromosome breaks in man."

These findings on drosophila have been supported by two other studies where the concentration of LSD ranged from 0.28 to 100 μg/ml (*61, 62*). In a study with a different standard genetic system, fungus (*Ophistoma multiannulatum*) cells were exposed to 20 to 50 μg of LSD per milliliter. There was no difference between treated and control cells (*63*).

When drosophila were injected with 2,000 to 10,000 μg of LSD per milliliter, significant increases in lethal mutations were found (*62, 64*). The evidence of no effect from 0.28 to 500 μg/ml and a definite effect from 2,000 to 10,000 μg/ml is consistent with a threshold dose response (*65*), or a sigmoid dose-effect relation.

When LSD was ingested, doses 18 times greater (8460 μg per gram of body weight) than those injected were required to induce equivalent numbers of lethal mutations (*62*). Positive mutagenic effects were obtained by injection only at or above 470 μg/g. On the basis of body weight, this would be roughly equivalent to a dose of 30×10^6 μg of LSD in a 70-kg man.

The data on drosophila and fungi suggest that LSD is a weak mutagen effective only in extremely high doses and that it is unlikely to be mutagenic in any concentration used by human subjects.

LSD and DNA

Early reports of an LSD-chromosome interaction suggested the need for direct observation of the effect of LSD on DNA. Yielding and Sterglanz (*66*) performed spectrophotometric studies on the binding of the active and inactive optical isomers of LSD and a hallucinogenically inactive analog (*dl*-2-Br-lysergic acid diethylamide) to calf thymus DNA and found that the patterns of optical absorption were similar. They suggested that binding to DNA might be a general property of this group of compounds. Binding did not take place with yeast RNA or nonhelical DNA, suggesting that binding is specific for helical DNA. They interpreted their data as showing that each nucleotide residue in helical DNA is a potential binding site.

Wagner (*67*) described LSD as a planar, cationic, aromatic molecule that has the molecular characteristics necessary for interaction with the phosphate anions and for stacking between the bases of the DNA helix. He reported circular dichroism experiments which suggested that the specific mechanism of action of LSD on DNA was a direct interaction, by intercalation of LSD within the DNA helix, causing conformational changes that appeared unlikely to result in a decrease of internal stability sufficient to cause breakage of chromosomes. Smythies and Antun (*68*) performed similar experiments which supported the hypothesis that LSD binds to nucleic acids by intercalation.

We agree that the interaction between LSD and DNA is inadequate to explain the claimed chromosome breaking potential of LSD. However, the evidence of LSD intercalation into the DNA helix seems to provide the physical mechanism for the mutagenic effects of high doses in drosophila and the fungus, as reviewed above.

Is LSD a Teratogen?

Rodents have been used to study the relation between LSD and congenital malformations, fetal wastage, and germinal chromosome mutation. Data from humans and primates are scanty.

Auerbach and Rugowski (*69*) reported that low doses of LSD administered early in gestation (0.05 to 1.0 μg, days 6 to 7) induced a high rate of embryonic malformations in several strains of mice (BALB/CAu, C57BL6/Au, C3H/HeAu, and BALB/C×57BL). Central nervous system abnormalities were common, but there was no observable effect of LSD exposure occurring later than day 7 of gestation.

In another study (*70*) with Swiss-Webster mice at higher doses, a high incidence of lens abnormalities was reported, although malformations of the central nervous system were not found even on histologic examination. In another study (*12*) the frequency of malformed embryos in A/Cum mice was 19 percent compared to 10 percent in controls (at doses 25 to 1000 times the usual human dose), suggestive of a potentiation of individual threshold differences. In this same study NIH general purpose mice that received equivalent doses showed no teratogenic effect, suggesting strain specificity. In still another (*71*) in which high doses (500 μg/kg per day) were administered to pregnant Swiss mice no abortifacient, teratogenic, or embryonic growth depressing effects were observed in 521 offspring.

An autoradiographic study (*72*) on pregnant Yale Swiss mice showed that ^{14}C-labeled LSD easily penetrated cell membranes, rapidly crossed the placental barrier, and entered the fetal circulation. When given during early pregnancy, 2.3 percent of the ^{14}C-labeled LSD traversed the placenta within 5 minutes, compared to only 0.5 percent of that given late in gestation.

It has been suggested that a teratogenic effect might occur through direct chromosomal damage to germ cells (*6*). Such damage could only be ascertained by direct observation of germinal cells from gonadal biopsy. A study (*73*) on meiotic chromosomes of male mice (strain was not reported) revealed a small number of breaks, gaps, and fragments in treated animals—statistically not significant—at massive doses (1000 to 8000 μg of LSD). Positive indications of meiotic damage to the male of the C3H He/Ha strain were seen in another study at doses (25 μg/kg) that were six to eight times the usual human dose on the basis of body weight (*74*). Both of these studies examined male meiosis only. No evidence of structural change in meiotic chromosomes or impairment of meiotic activity was found by a third team of investigators (*75*) who administered a single dose (27 to 30 μg of LSD) or a series of doses (0.1 to 5.0 μg of LSD per day) for 8 to 31 days to male and female mice of another strain (Imperial Chemical Industries–derived). Yet the doses used were 90 times greater than those shown to be teratogenic for several strains of mice (*69*).

Stunted and stillborn offspring were reported (*76*) in Wistar rats if given a single subcutaneous dose (5 μg of LSD per kilogram of body weight) early in gestation (day 4), but not late (after day 7). This study was performed on only ten mothers with 85 offspring. In repeating their work (*77*) on 260 pregnant rats and 1800 offspring, these in-

vestigators found damage to LSD-treated litters that was three to four times higher than in controls. The proportion of deaths during gestation, absorption, resorption, runting, stillbirths, and offspring mortality was increased over that of the controls. The LSD effects appeared to be dose-related and persisted into the second generation. Teratogenicity during any particular organogenetic period was not demonstrated. There are four other studies (*71, 78–80*) on Wistar rats which involved: very large numbers of offspring (approximately 1500); extremely high doses (100 to 6000 μg); meticulous repeats (same dose, day, species, and route of administration); histologic examination; maze running, shock avoidance, and food competition (*79, 80*). Very high doses of LSD were found to disrupt pregnancy (*79*). This effect was dose-related. The median effective dose (ED_{50}) at which 50 percent of the females produced no viable births was 4760 μg of LSD per kilogram of body weight. In all four studies no teratogenic effects in the offspring were reported (Table 5).

A study (*81*) in which hamsters received a wide range of doses of LSD (0.84 to 240 μg) on day 8 of pregnancy revealed 5 to 8 percent malformations of the brain, spinal cord, liver, and other organs. Some fetuses had several malformations, and the actual percentage of malformed fetuses was not specified. No correlation existed between dose and number of malformations. These findings have not been confirmed when higher doses (10 to 500 μg/kg) were used (*12, 71*). Diapolo (*12*) suggested that, in the case of a truly positive teratogenic effect, a dose-response relation and an increase in abnormalities much greater than 5 to 8 percent (that is, less than one animal per litter could have been malformed) would be expected. No teratogenic effect was shown in rabbits given doses of 20 and 100 μg of LSD per kilogram of body weight on days 4 to 12 of gestation (*82*).

An overall view of the studies on rodents indicates a wide variation of individual, strain, and species susceptibility to the effects of LSD. The effect, when found, was at a highly specific time early in gestation. No effect was reported when exposure occurred late in pregnancy. Cohen *et al.* (*27*) have suggested that if a similar critical period exists early in human pregnancy abortions would result in the majority of instances, rather than full-term delivery of malformed infants.

Studies of rodents indicate what might possibly occur in humans. However, the fetal growth and development in these species is different from that in man. For example, placental function differs, particularly in the degree of intimacy between fetal and maternal circulations. The higher rodents show the nearest approach to actual intermingling of the blood of the two circulations (the hemoendothelial placenta). In humans there are chorionic villi (hemochorial placenta) which, in rodents, reduce to bare blood vessels whose endothelial walls alone separate the fetal blood from the maternal sinuses (*83*). For this reason alone, rodents are more sensitive than humans to the teratogenic potential of any given substance. Auerbach considers direct ". . . extrapolation from mice to man to be hazardous" (*84*).

Kato *et al.* (*51*) reported the effects of multiple (from 4 to 11) subcutaneous injections of LSD in pregnant rhesus monkeys. Single doses of 0.125 to 1.0 mg/kg (total dose: 0.875 to 9.0 mg/kg) were administered to four monkeys, starting at months 3 to 4 of gestation. Of the four treated animals, one delivered a normal infant, two were stillborn with facial deformities, and one died at the age of 1 month. Two control animals delivered normal offspring. The lowest dose exceeded by 100-fold the usual experimental dose in man. No conclusions were offered from the foregoing data.

The information on humans is meager. In three studies ingestion of illicit LSD by pregnant women resulted in elevated numbers of chromosome breaks in their offspring (*26, 27, 31*). Of the 14 children exposed to illicit LSD in utero, 10 had elevated breakage rates. In the one child studied serially the chromosome breakage rate became normal (*31*). We have suggested that these findings are relevant to the effects of drug abuse in general rather than to the effects of pure LSD. All of these children were in good health and had no birth defects.

There are six cases of malformed infants born to women who used illicit LSD prior to or during pregnancy. Zellweger *et al.* (*85*) reported the birth of an infant with a malformed right leg (unilateral fibular aplastic syndrome) to a woman who used illicit LSD four times during the first trimester. Hecht *et al.* (*86*) reported an infant with a malformed arm born to a woman who had been exposed to illicit LSD, cannabis, and several antiemetic drugs during the first trimester. Carakushansky *et al.* (*87*) reported an infant with a terminal transverse deficit of portions of fingers of the left hand and syndactyly of the right hand, whose mother was exposed to illicit LSD and cannabis during pregnancy. Assemany *et al.* (*88*) reported an infant with amputation deformities of the third finger of the right hand and the third toe of the left foot. The mother had been taking unknown amounts of illicit LSD before and throughout pregnancy. Hsu *et al.* (*89*) reported an infant girl born with trisomy-13 to parents who used illicit LSD prior to but not during pregnancy. Hsu *et al.* suggested that LSD could have damaged the germ cells prior to pregnancy. However, the mother was using cannabis, barbiturates, and amphetamines during pregnancy. We suggest that the transplacental effects of these compounds cannot be discounted. Eller and Morton (*90*) reported an infant with severe deformities born to a woman who had ingested illicit LSD once near the time of conception. An estrogen and medroxyprogesterone were ingested in the first trimester. Eller and Morton stated that previously described infants with this combination of anomalies, referred to as spondylothoracic dysplasia, were usually the result of consanguinous marriages, and they stated that the cause probably involves an autosomal recessive mode of inheritance.

In all six reported cases the drug was illicit. There is no report of congenital malformations in human offspring exposed before birth to pure LSD.

McGlothlin *et al.* (*91*) reported frequencies of spontaneous abortions, premature births, and children with birth defects in 148 pregnancies when one or both parents were exposed to pure and illicit LSD or to pure LSD alone. The only increased risk observed was in spontaneous abortions in the group exposed to both pure and illicit LSD (37 percent), as compared to the group exposed to pure LSD (15 percent) and the general population (20 percent). Only 12 pregnancies involved ingestion of LSD (three pure and nine pure plus illicit) during gestation. Six of these ended in abortions (one pure and five pure plus illicit). McGlothlin *et al.* noted that one woman accounted for five of the ten abortions in the pure plus illicit group and these five were

five of the six abortions involving exposure during pregnancy. This same woman had one abortion prior to LSD use. If this subject is not included in the calculations, the spontaneous abortion rate is 24 percent in the group exposed to pure and illicit LSD before pregnancy and 14.4 percent for exposure during pregnancy.

In the only study of human or primate germ cells exposed to LSD, Hulten *et al.* (*31*) performed a testicular biopsy and found no evidence of an increased rate of meiotic chromosome aberrations 6 months after the last ingestion of a very large amount of illicit LSD and other drugs.

In summary, then, a teratogenic effect has been reported in hamsters, rats, and mice, but only the data on mice have been confirmed. The information from lower primates, although preliminary, is suggestive of a teratogenic effect and deserves further investigation. Case reports of malformed children born to users of illicit LSD are rare, although there is some indication of an increased risk of spontaneous abortion. There is no evidence that pure LSD causes birth defects or fetal wastage in man.

Summary and Conclusion

Of nine studies in vitro, six have indicated some degree of induced chromosomal breakage after exposure to LSD; three failed to confirm these results. The damage, when found, was generally of the chromatid type, arising during or after DNA synthesis. This damage, with one exception, was the result of concentrations of drug and durations of exposure which could not be achieved in humans with reasonable dosages. There did not appear to be a dose-response relation. The magnitude of damage, when found, was in the range encompassing the effects of many commonly used substances. The absence in vitro of excretory and detoxifying systems present in vivo, as well as several negative reports, cast doubt on the relevance of in vitro results.

In 21 chromosomal studies in vivo, 310 subjects were examined. Of these, 126 were treated with pure LSD; the other 184 were exposed to illicit, "alleged" LSD. A maximum of only 18 of 126 (14.29 percent) of the subjects in the group exposed to pure LSD showed higher frequency of chromosome aberration than the controls. In contrast, a maximum of 90 of 184 (48.91 percent) of the subjects taking illicit LSD showed an increase in frequency of aberrations. Of all the subjects reported to have chromosome damage, only 18 of the 108 (16.67 percent) were exposed to pure LSD. The frequency of individuals with chromosomal damage reported among illicit drug users was more than triple that associated with the use of pharmacologically pure LSD. We conclude that chromosome damage, when found, was related to the effects of drug abuse in general and not, as initially reported, to LSD alone. We believe that pure LSD ingested in moderate dosages does not produce chromosome damage detectable by available methods.

No significant work on carcinogenic potential of LSD has been reported so far. No cause-and-effect relation and no increase in the incidence of neoplasia among LSD users have been demonstrated. Case reports (three in 4.0 years) of leukemia and other neoplasia in this population are rare.

The results of early chromosome studies suggested that true genetic damage might be a consequence of LSD exposure. The comprehensive evidence from studies on drosophila indicates no mutagenic effect from 0.28 to 500 μg of LSD per milliliter and a definite mutagenic effect from 2,000 to 10,000 μg/ml; this is consistent with a threshold response or a sigmoid dose-effect relation. We believe that LSD is, in fact, a weak mutagen, effective only in extremely high doses; it is unlikely to be mutagenic in any concentration used by human subjects.

Circular dichroism experiments suggested that the specific mechanism of action of LSD on DNA may be a direct interaction resulting in conformational changes in the DNA helix. These changes are unlikely to result in a decrease of internal stability sufficient to cause breakage of chromosomes, but they may be the physical basis of the weak mutagenicity.

Early chromosomal studies implicated LSD as a potential cause of congenital malformations, fetal wastage, and germinal chromosome damage. First reports of a teratogenic effect in hamsters and rats have not been confirmed. A review of 15 rodent studies indicated a wide range of individual, strain, and species susceptibility to the effects of LSD. The applicability of such investigations to man is doubtful. In a study of human pregnancies, those exposed to illicit LSD had an elevated rate of spontaneous abortions. There is no reported instance of a malformed child born to a woman who ingested pure LSD; there are six cases of malformation associated with exposure to illicit LSD, four of which have similar limb defects. Given, however, the high frequency of unexplained "spontaneous" birth defects, the rare occurrence of malformed infants born to women who used illicit LSD may be coincidental. While there is no evidence that pure LSD is teratogenic in man, the use of any drug during pregnancy requires that its potential benefits significantly outweigh its potential hazards.

From our own work and from a review of the literature, we believe that pure LSD ingested in moderate doses does not damage chromosomes in vivo, does not cause detectable genetic damage, and is not a teratogen or a carcinogen in man. Within these bounds, therefore, we suggest that, other than during pregnancy, there is no present contraindication to the continued controlled experimental use of pure LSD.

Note added in proof: A brief review has been brought to our attention. Although based on a sample of only 15 studies the author reached conclusions similar to our own (*92*).

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Polyacrylamide Gel Electrophoresis

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Fractionation of proteins, nucleic acids, and other charged macromolecules has generally required successive use of several fractionation steps, one sensitive primarily to molecular size (such as gel filtration) and another based mainly on molecular net charge (such as free electrophoresis, ion exchange chromotography). Zone electrophoresis in polyacrylamide gel (*1, 2*), designated as polyacrylamide gel electrophoresis (PAGE), simultaneously exploits differences in molecular size and charge for purposes of fractionation.

Range of applicability. The synthetic polymer, polyacrylamide, can be made to provide an effective median pore radius of 0.5 to 3 nm (*3*) by the simple device of adjusting the total acrylamide concentration, designated (*4*) $\%T$ (3 to 30% w/v) (*5, 6*), and the concentration of cross-linking agent, designated (*4*) $\%C$ (1 to 25% of total monomer) in the polymerization reaction (Fig. 1). Larger pore sizes can be produced when the polymer is stabilized by agarose (*7–9*). The pore sizes can be selected for optimal resolution between any two species (*10*). The wide range of applicability of PAGE is illustrated by the fractionation of oligonucleotides (M.W. < 1000) (*5*) and high-molecular-weight RNA (M.W. $> 10^6$) (*6, 8, 9*). One can also optimize "charge separation" by operating at any *p*H between 3 and 11 to provide the maximal difference be-

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